The Development of the Sea Anemone Bolocera Tuediae (Johnst.).

By

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With Plate 22.

Bolocera tuediae was recorded and described from deep water near Berwick by Johnston (**11**) in 1832. Gosse described it more fully in 1860 (**10**, p. 185) and the following is his summary of its characters : 'Base adherent, not much exceeding the column. Column pillar-like, the diameter and height sub-equal ; surface generally very smooth, studded with warts remotely scattered. Disc smooth, circular in outline, not overlapping the column. Tentacles short, thick, constricted at foot, obtusely pointed, longitudinally furrowed, flexuous and motile, easily separated, not retractile. Mouth raised on a cone. Stomach capable of being greatly protruded.' The tentacles are, however, moderately long and slender when fully extended during life.

Carlgren (**3**, pp. 34–6) adds that the genus Bolocera is characterized by the presence of a relatively well-developed diffuse or circumscribed endodermal sphincter, that the column has no ectodermal longitudinal muscular layer, that the tentacles have a well-marked endodermal sphincter at their bases, and that probably all the mesenteries except the eight ' Edwardsia ' ones are fertile. Carlgren follows McMurrich (**16**) in judging that Bolocera must be placed in a separate Family, the Boloceridae. Its nearest allies are probably among the Antheinae in which, however, the sphincter is extremely feeble if not entirely absent (see Delage, **6**, ii. 2, pp. 503–5).

In the Clyde Fauna List (Laurie, **18**, p. 367) Bolocera

tuediae is put down as occurring at depths of from fifteen
to seventy-five fathoms. My own records lower the first limit
to thirty fathoms. While possessing an attaching base and
capable of adhering weakly to the sides or bottom of an
aquarium tank, Bolocera appears to live usually on muddy
bottoms, and is almost always brought up by itself when
taken with the dredge or on the long lines of fishermen. It
has great stinging powers, and one has to risk a somewhat
severe urticaria when handling it alive.

The sexes are separate and the gonads are at their largest
in the end of February and beginning of March. Unfortunately
the females very seldom spawn in captivity. The eggs are
retained and undergo absorption after a time. Probably
want of the natural food is a contributing reason. The males
shed their sperm more freely.

Only a few eggs were obtained in 1916 and 1917, but in
March 1918 large numbers were extruded by a recently-taken
specimen. These after floating about in the Bolocera tank
were duly fertilized, although none of the males at the time
had emitted a noticeable amount of sperm.

Maturation must take place just prior to extrusion. Serial
sections of full-sized ovaries show the eggs with large-sized
germinal vesicles, but in similar sections of freshly-shed unfer-
tilized eggs the nucleus is so small and inconspicuous that
I could not detect it.

The eggs are spherical, 1·1 mm. in diameter, and pink or
flesh coloured, i.e. of much the same tint as the animal itself.
They tend to float, and when floating show no polarity as
regards upper and under sides. They are surrounded by
a membrane beset all round by small conical bunches of spines.
The interior is crowded with small granules faintly stainable
with haematoxylin, small yolk-spheres staining red with
eosin, and large clear spherules unaffected by re-agents, the
latter being relatively more numerous towards the centre of
the egg. In certain methods of preservation (e.g. corrosive
sublimate followed by graded alcohols) an inner core, about half
the diameter of the egg, tends to become separated from the

outer zone. Just under the egg-membrane is a thin layer where the first-named granules are very numerous, the clear spherules absent, and the yolk-spheres few in number.

Bolocera has the largest eggs of all the Clyde Anemones I have investigated. Their diameter, 1·1 mm., compares with 0·1 mm. for Metridium dianthus, 0·3 mm. for Anthea cereus, 0·1 mm. for Sagartia, 0·25 mm. for Adamsia palliata, 0·6 mm. for Urticina coriacea (the shore Urticina), 0·7 mm. for Urticina crassicornis (the submerged Urticina). Full-grown ovarian eggs of Gonactinia prolifera and of Actinia equina measure respectively 0·07 and 0·15 mm. in diameter. The Bolocera egg-membrane and its spines resemble but are hardly so strong as those of Urticina. The egg-contents of the two are much the same. Anthea (and Actinia equina, according to Lacaze Duthiers) has spiny egg-membranes, but in Metridium, Sagartia, and Adamsia the membranes in question are smooth.

In Bolocera, as in Urticina (Appellöf, **1**), the fertilized nucleus gives rise to a number of daughter nuclei (sixteen in Urticina) before the egg-mass undergoes cleavage. In particular cases I have estimated the number as not less than twenty-four. The fertilized nucleus probably lay at a point somewhere in the deeper layer of the outer zone, about a third of the diameter of the egg inwards from the surface. The daughter nuclei, as they increase in number, spread laterally at this level from the point in question until they are more or less equally distributed all round. In the egg illustrated by fig. 1, eight nuclei were present, all of them in one hemisphere.

Slightly older eggs examined under reflected light begin to show rounded bosses or humpings which appear first at one side (no doubt the side towards which the fertilized nucleus lay), and afterwards extend all over the egg-surface. They soon become better defined and separated from one another by linear furrows. Segmentation of the egg-mass is in progress, and serial sections show that each hump is the outer end of a large more or less conical cell the apex of which is directed centrally. The whole egg increases slightly in size, and a small

central cavity filled with coagulable fluid makes its appearance.
The egg-membrane is not separated off as a membrane of
fertilization, but is found to follow closely every surface
change of contour so long as it is recognizable. As segmentation
proceeds, non-nucleated portions separate off from the inner ends
of the cells, and, mixing with the blastocoelic fluid, form a central
diffuse trophenchyme. At this stage one or two whole cells
may share the same fate by migrating or getting pushed
inwards from the surface. Their nuclei proliferate ; but,
soon losing control over the cell-contents which become
trophenchymal, are destined to degenerate along with the
other trophenchymal nuclei to be described later.

A little later the Bolocera egg shows very markedly those
peculiar surface grooves and foldings which Masterman
first described in the case of Cribrella (**17,** p. 8), and which have
since been noted in many ova (**8,** p. 12). During this process there
is a tendency, better marked in some instances than in others,
for the egg to assume the form of a flattened disc the edges of
which become turned upwards like those of a saucer. The
surface grooves and the saucer cavity gradually fill out, so
that the egg becomes almost spherical again. The saucer
cavity is accordingly not the archenteron, though gastrulation,
which soon supervenes, affects the part of the egg-wall
which was formerly the hollow of the saucer. In the fully-
formed blastula this part often remains flat while the rest of
the blastula wall is spherical.

An important point to note is that as the surface folds smooth
out, many single cells and groups of cells are nipped off from
the recesses, and migrating inwards become included within
the trophenchyme. I thought at first that these cells were
going to form the endoderm of the larva. But this is not so.
Their cell outlines will disappear and their nuclei degenerate.

Gastrulation.—In typical cases (see e.g. figs. 7–9)
a relatively large portion of the blastula wall shows
flattening and sinks gradually downwards, the margins of
this portion closing in slowly to form the lip of the blasto-
poric opening. At the same time this lip becomes slightly

involuted giving rise to the rudiment of the stomodoeal canal.

The invaginating area soon presses against the trophenchyme, and we often find at this stage secondary flattening of the whole egg and foldings of its walls, which are probably caused by the resistance of the trophenchyme to the progress of invagination. However, in course of time, the trophenchyme finds its way through the inpushing endoderm into the cavity of the archenteron. First, the fluid and fine granules begin to get through, then the yolk-spheres, and lastly the clear spherules. The process appears to be mechanical in the sense that the trophic material passes through interstices between endoderm cells, and is not first swallowed or assimilated and then excreted into the archenteron.

As gastrulation proceeds most of the trophenchymal nuclei disintegrate, but some pass with the trophenchyme into the archenteron and are absorbed later.

It is of particular interest to note that in a few cases the end-result of gastrulation is attained by a process which may be described more accurately as unipolar immigration than as invagination. In such cases the cells over a relatively small area at one pole of the blastula begin to sink inwards through the trophenchyme, at the same time proliferating and spreading out so as almost to lose their continuity with one another. This process continues until having passed through the whole depth of the trophenchyme, they abut against the ectoderm where they soon form a continuous sheet of endoderm lining an archenteric cavity which now naturally contains all the trophenchyme. Sometimes the process is intermediate between that described above and open invagination. Similar differences occur among the eggs of different Crustacea, but not so far as I know among the eggs of the same Crustacean species. We may put down the variations in Bolocera as probably due to differences in the character of the yolk, noting that those blastulae which showed the fewest foldings and the least deformation tend also to form their endoderm by unipolar immigration.

A mesogloeal sheet only begins to form after the ectoderm and endoderm have come in contact. Accordingly it appears first at the oral end of the larva. Both layers seem to take part in its formation.

Comparison with other Anemones as regards the Stages up to the end of gastrulation.

Metridium dianthus.—Nuclear division and segmentation go together from the first ; blastula with a hollow central cavity ; endoderm formed by invagination (Gemmill, **9**). McMurrich, however, stated (**15**) that the endoderm is formed by delamination. Sagartia troglodytes.—As in Metridium. Adamsia palliata.—Cleavage begins after the second nuclear division ; the preblastula is a wrinkled disc, becoming saucer-shaped, and then smooth and spherical or oval ; the inner yolky ends of the cells separate off to form a central trophenchyme normally without nuclei ; gastrulation is by invagination (Gemmill, **9**), and the trophenchyme passes through the inpushing endoderm into the archenteron. Faurot (**7**), however, stated that the endoderm is formed by delamination. Urticina crassicornis.—Development is much the same as in Bolocera. Cleavage, however, begins when there are sixteen nuclei in the egg, and the trophenchyme nuclei are sparing or absent. The crumpling and folding of the wall of the early blastula which I find to be very well marked in the eggs of Urticina have not been described by Appellöf in his otherwise excellent account of the development of this species (**1**). Actinia bermudensis.—Early stages not determined ; gastrulation by invagination (Cary, **5**). Actinia equina.—Early stages not determined ; endoderm formation by invagination according to Jourdan (**12**), but by immigration or delamination according to Appellöf (**1**), who states that the mouth opening is a secondary break-through.[1] Cerianthus and an Actinian allied to A. equina.—Endoderm formation by invagination (Kowalevsky, **13**).

Movements.—Cilia are acquired during the middle blastula

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[1] My own observations (Millport, 1920) are entirely in favour of the open invagination method of endoderm formation in this species.

stage and show activity before the egg-membrane spines have disappeared. Blastulae and early gastrulae move irregularly, but late gastrulae and older larvae progress with the aboral end in advance, rotating at the same time in the contra-solar direction as viewed from this end. Meantime a change of specific gravity has occurred and the larvae tend to remain on or near the bottom. Elongation of the larva has also taken place in the oral-aboral axis. The shape now varies according to contraction but is usually pyriform, the aboral end being the smaller. Over this end the ectodermal cells elongate, becoming clear at their outer extremities. They are preparing a cement in view of fixation. At no stage is there present a specially elongated tuft of cilia such as is characteristic of the larvae of Metridium and Sagartia and in a less degree of Actinia equina.

Mesenteries.—The eight primary or Edwardsia mesenteries appear, first in the neighbourhood of the mouth, as folds of the endoderm, each containing a thin mesogloeal sheet continuous with the general mesogloeal layer between ectoderm and endoderm. The sulco-laterals (ventro-laterals) are the first to develop. The remainder appear practically simultaneously, but I could sometimes make out that the sulculo-laterals were a little ahead of the sulcar directives, and the latter of the sulcular directives. In the figures the mesenteries are numbered 1, 2, 3, 4, corresponding to the above sequence.

All the primary mesenteries have appeared prior to fixation, and at this stage the oral ends of the sulco-laterals are already edged by a down-growth of stomodoeal ectoderm for the mesenteric filaments, and project so far inwards as almost to meet one another. The developing muscle banners on all the mesenteries show the characteristic Edwardsia arrangement.

Fixation occurs about twenty-five days after shedding of the eggs, and is at first by cement attachment, the larvae adhering usually to the bottom but sometimes to the sides of the hatching vessel. The base, at first small and pointed, soon becomes larger and disc-like. Shortening of the larva takes place till the length of the column is less than its breadth ; the oral surface flattens ; the mouth opens widely and elongates

in the axis of the directive mesenteries. Then the young anemones remain quiescent except in showing the following changes.

1. Absorption of the trophenchyme within the archenteron. It is partly used up and partly absorbed into the endoderm layer, which becomes greatly thickened, as well as extended by the fuller growth of the mesenteries.

2. Down-growth of stomodoeal ectoderm to form mesenteric filaments on the sulculo-lateral mesenteries. This began prior to fixation on the sulco-laterals.

3. Formation of a new mesentery in each lateral and sulco-lateral Edwardsia space. These mesenteries can be detected near the middle of the column of the larva earlier than near the mouth or on the base. In my oldest specimens their developing muscle banners could with much difficulty be made out, each being formed on the sulcular side of its mesentery as in Urticina (**1**). They are thus suitably placed to form with the Edwardsia sulco- and sulculo-laterals, the primary hexactinian ulco- and sulculo-lateral mesenteric pairs on each side, the remaining pairs being of course the sulcar and sulcular directives (fig. 15).

I tried to rear the young anemones further, but so far without success, although I gave the larvae the chance of settling down on shells, stones, glass, and mud, and of living after attachment either in separate hatching vessels, or in a tank with sea-water circulation. Those which settled on mud retained a rounded base, but otherwise reached much the same stages as the attached ones. None went the length of growing out tentacles. The attached specimens were less firmly fixed, and yet crept about less freely, than the corresponding stages in Urticina, in which also, as was shown by serial sections, the mesoglocal and muscular tissues were more strongly developed.

For further comparative details and a discussion of some general problems connected with coelenterate development, reference may be made to a recent paper by the author in the ' Phil. Trans. Roy. Soc. Lond.' (**9**) on the development of Metridium dianthus and Adamsia palliata.

Summary and Chronology.

Egg large, floating ; maturation prior to shedding ; fertilization external ; at least twenty-four nuclei present before cleavage of egg-mass takes place (fifteen hours) ; cleavage total leaving a small central cavity ; the inner ends of the cells separate off to form a central trophenchyme (twenty-four hours) ; a greatly-folded ' preblastula ' stage during which groups of cells are included in the trophenchyme (forty-eight hours) ; the blastula becomes more or less smooth and spherical (three to three and a half days) ; gastrulation begins (four to five days) ; gastrulation complete and first mesogloea formed (six and a half to seven and a half days), the trophenchyme passing into the archenteron, and degeneration of its nuclei taking place ; blastopore narrows and virtually closes, involution of stomodaeum taking place ; larva elongates (nine to ten days) ; sulco-lateral mesenteries begin to form (fifteen days) ; aboral end shows cement gland formation, and rudiments of the other mesenteries appear (twenty days) ; fixation and shortening of the larva (twenty-five days) ; formation of four additional mesenteries (thirty-six days) ; complete absorption of trophenchyme within archenteron (thirty-six days). For cilia, movements, &c., see p. 582. At no stage is there a tuft of specially elongated aboral cilia.

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EXPLANATION OF FIGURES.

bl.c., blastocoele cavity ; *end.*, endoderm ; *g.*, commencement of gastrulation ; *n.f.*, furrows on blastula from which trophenchyme nuclei are nipped off ; *n.tr.*, these nuclei degenerating within the trophenchyme ; *st.*, stomodaeum ; *tr.*, trophenchyme ; *tr.a.*, trophenchyme within the archenteron ; 1–6, the primary hexactinian mesenteries numbered according to their order of development (see explanation in text).

Fig. 1.—Section of Bolocera egg, 10 hours after fertilization, showing five nuclei ; all the nuclei are in one hemisphere of the egg.

Fig. 2.—Section of egg about 18 hours after fertilization, showing the characteristic complete segmentation (see p. 579).

Fig. 3.—Similar section about 28 hours after fertilization. Note the passage of one of the cells inwards from the surface, and commencement of trophenchyme formation.

Fig. 4.—Similar section about 36 hours after fertilization.

Fig. 5.—Similar section about 48 hours after fertilization. Note the extremely folded and crumpled surface (see p. 580).

Fig. 6.—Similar section about $3\frac{1}{2}$ days after fertilization. The folds have mostly straightened out leaving behind numerous groups of cells nipped off from their recesses and enclosed within the trophenchyme. The outlines of these cells disappear and the nuclei degenerate now or later.

Fig. 7.—Similar section about $4\frac{1}{2}$ days after fertilization. Commencement of gastrulation.

Fig. 8.—Similar section about $5\frac{1}{2}$ days after fertilization.

Fig. 9.—Similar section about 7 days after fertilization.

Fig. 10.—Similar section about 8 days after fertilization, showing (a) the progress of gastrulation, (b) the passage of the trophenchyme through the inpushing endoderm into the archenteron, and (c) the involution of the lips of the blastopore to form the stomodaeum.

Fig. 11.—Longitudinal section of larva 12 days old. The shape is now pyriform and the cells at the aboral end are becoming elongated and glandular.

Fig. 12.—Transverse section across larva 15 days old near its oral extremity showing the two first mesenteries—the sulco-laterals.

Fig. 13.—Transverse section through larva 20 days old showing formation of all the Edwardsia mesenteries, viz. (1) the sulco-laterals ; (2) the sulculo-laterals ; (3) the sulcar directives, and (4) the sulcular directives. In this specimen the last named are the smallest and were no doubt the latest to appear (see p. 583). The sulco-laterals are now edged by a down-growth of epiblast for the mesenteric filaments.

Fig. 14.—Diagram of transverse section of attached specimen (25 days old) to illustrate the arrangement of the eight Edwardsia mesenteries which are numbered as in the previous figure, and on which the rudiments of muscle banners can now be made out.

Fig. 15.—Similar transverse section of attached specimen (36 days old) in which a new mesentery (numbered 5 and 6 respectively) has developed in each sulco-lateral and lateral Edwardsia space. Muscle banners are beginning to develop on their sulcular sides. The six primary hexactinian mesenteric pairs will consist of the sulcar directives, the sulcular directives, two pairs made up of two and five on either side, and two pairs made up of one and six on either side.